The isoepoxydon dehydrogenase gene of the patulin biosynthetic pathway, patulin detection, and the utility of species names in patulin-producing fungi

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Summary

Isolates (20) of fungi representing 13 species and 5 genera were analysed for the isoepoxydon dehydrogenase gene and patulin detection to provide further information on the distribution of these traits. Strains identified as members of patulin producing species may not even have the potential for patulin production due to a lack of the key IDH gene. This demonstrates the advantages of the biochemical analyses described herein with ramifications for preserving fungi.

Keywords: Fungi; Patulin; Isoepoxydon dehydrogenase

Introduction

Patulin (Fig. 1) is a very toxic mycotoxin associated with *Penicillium expansum* infection of apples, and other commodities and fungi. New EU regulations on maximum concentrations are being proposed in food and drink (Jones and Toal, 2003). Other commodities [e.g. water (Kelley *et al.*, 2003)] are being considered from a mycotoxin perspective. Patulin is produced via a polyketide metabolic pathway in which the conversion of isoepoxydon to phyllostine (Fig. 2) is a unique and late step. It constitutes part of a differentiated pathway. A probe for the isoepoxydon dehydrogenase (IDH) gene was developed by which environmental samples Paterson *et al.* (2000) and isolated penicillia (Paterson *et al.*, 2000; 2003) were analysed. *P. brevicompactum* was

determined to be a potential producer of patulin from apple production systems in England although it was unrecorded previously as producing the mycotoxin. Varga *et al.* (2003) employed the gene probe to investigate patulin production, amongst other things, in *Aspergillus* section *Clavati*. The first report of patulin from *A. longivesica* was recorded, and variation in the possession of the gene and patulin production in *A. clavatus* was reported. It is important to be able to detect all sources of patulin contamination accurately in commodity systems.

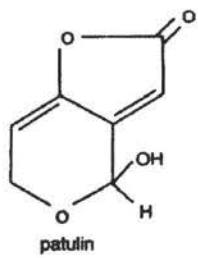

Figure 1. Chemical structure of patulin.

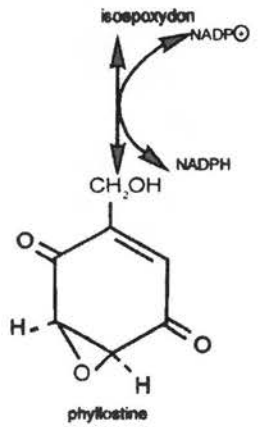

Figure 2. The conversion of isoepoxydon to phyllostine mediated by IDH.

Previous work (*e.g.* Wang *et al.*, 1991) on the regulation of patulin in *P. urticae* (syn. *P. griseofulvum*) will be more applicable to other fungi if the genetics are similar, and may be useful in controlling patulin concentrations in commodities. Assessments of the evolutionary significance of patulin production within fungi will be able to be made more readily (Varga *et al.*, 2003). Finally, the information will be of use

in preserving patulin production in fungi, and other biochemical characters by analogy. The present paper reports on the occurrence of the IDH gene and patulin production in more taxa to extend knowledge of this capability and compiles the recent data on the subject to extend information to 243 strains representing 36 species and 6 genera.

Material and Methods

Fungi were analysed by the method of Paterson *et al.* (2000). Conventional PCR and electrophoresis methods were employed. The primers had the following sequence: 5′-CAATGTGTCGTACTGTGC CC-3′, and 5′-ACCTTCAGTCGCTGTTCCTC-3′. Patulin production was detected using the agar plug and TLC method. Visualisation procedure was spraying TLC plates with 0.5 % 3-methyl-2-benzo thiazoline hydrazone hydrochloride/water (w/v).

Results

Figures 3 and 4 demonstrate a gel of the IDH probe product from *P. expansum* and *P. roqueforti,* and TLC of patulin from *P. expansum*,

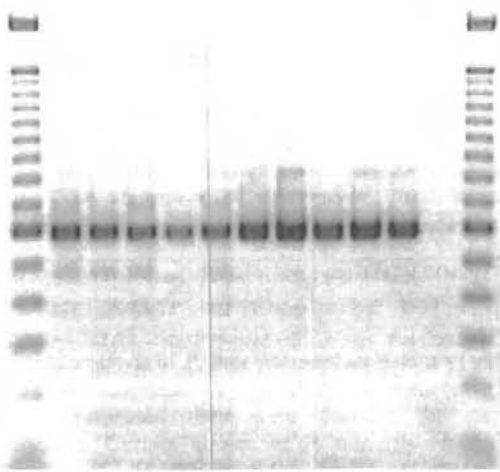

Figure 3. IDH gene fragment from *Penicillium expansum* (the first five tracks) and *P. roqueforti* (the remaining five tracks) excluding the extreme left and right marker tracks.

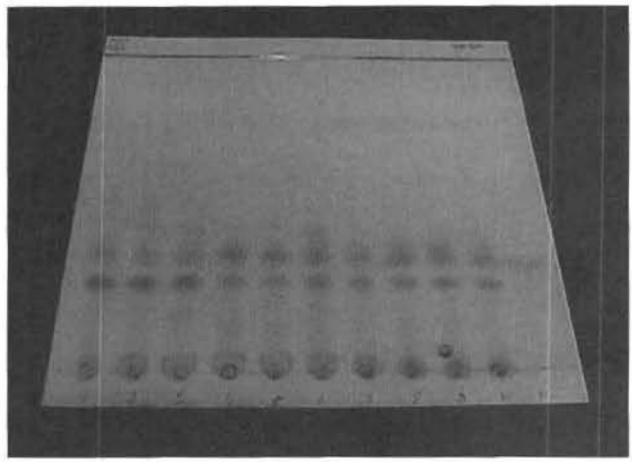

Figure 4. TLC plate of patulin from *Penicillium expansum* on malt extract agar. The extreme right tract is the yellow/brown patulin standard and the remaining tracts are from the fungus strains with patulin clearly detected.

respectively. Controls of A. *flavus* and *Ganoderma* were negative for the IDH gene fragment (Table 1). Sixty seven percent of the strains which contained the IDH gene fragment were positive for patulin detection. All strains which were negative for the gene were negative for patulin where tested.

Discussion

The presence of the gene fragment from *A. clavatus* was also reported in Varga *et al.* (2003). However, these authors reported that the IDH gene was not detected in some strains. Inconsistent patulin detection from different strains is also reported. Interestingly, patulin was observed from IMI 015949 (Table 1) but was not in Varga *et al.* (2003). This may reflect different analytical methods, cultures, preservation, etc. but illustrates that the gene probe is a more reliable indicator of patulin producing potential. The results for IMI 385435 were identical and confirm production despite the fungus being infected with a virus (Varga *et al.* 2003). *A. clavatus* was listed as positive for patulin production in Frisvad and Thrane (1993) and negative in Steinman *et*

Table 1. The isoepoxydon dehydrogenase gene and patulin detection from fungi. The number is the IMI identification

Fungi	IDH gene	Patulin detection
Aspergillus clavatus 015949; 358435	+	+
A. clavatus 232883	+	–
A. flavus 380661	–	not tested
A. giganteus 343711	+	+
A. giganteus 016154	+	–
Byssochlamys fulva 040021; 058422	–	–
B. nivea 361545	+	+
Ganoderma sp. 357185	–	–
Paecilomyces variotii 204127; 321342	–	–
Penicillium glandicola 321513	–	–
P. glandicola var. *glaucovenetum* 321511	+	–
P. griseofulvum 075832ii	+	+
P. griseofulvum var. *dipodomyicola* 296935	+	+
P. melinii 304279; 040216ii	–	–
P. novae-zeelandiae 040584ii	–	–
P. selandiae 304284	–	–

al. (1989). The gene was detected in two strains of *A. giganteus* and production in one. This species was negative for both traits in Varga *et al.* (2003) in the single strain investigated, and as a patulin producer in Frisvad and Thrane (1993). *A. terreus* was listed as producing patulin in Frisvad and Thrane (1993) but was not detected in Steinman *et al.* (1989). The IDH gene and patulin were not detected in the present study. *Byssochlamys fulva, Paecilomyces variotti, P. melinii, P. novae-zeelandiae* and *P. selandiae* were negative for the gene and detection, but were listed as producers in Frisvad and Thrane (1993). *P. glandicola* var. *glaucovenetum* was positive for the gene but negative for patulin detection, and was listed as positive in Frisvad and Thrane (1993). These differences within species can be explained by the gene being inconsistently found within a single species, as was the case for *P. brevicompactum* (Paterson *et al.*, 2003) and above. Isolates of *Paecilomyces* and *Byssochlamys* (and *Ganoderma*) were tested for the first time. The gene product from a *Byssochlamys* (*nivea*) is the first such report.

The data from Paterson *et al.* (2003), Varga *et al.* (2003) and the present work are combined and presented in Table 2. The occurrence of species with strains in more than one category is common. The most significant are species with representatives in IDH positive and negative, although genuinely non-producing strains but possessing the gene would be interesting. *P. expansum* and *P. roqueforti* are almost consistent in terms of possessing the gene. It would be interesting to analyse "domesticated" cheese producing *P. roqueforti* strains for the gene, and production. Most species which are assigned to one category require more strains to be analysed for confirmation. However, *P. simplicissimum* appears to be a genuine non-producing species. It might be assumed that individual strains would be consistent for possession of the gene. However, this requires to be determined experimentally.

A list of patulin negative strains not tested for the IDH gene is available in Paterson *et al.* (2003).

In conclusion, the patulin gene and production is distributed widely in fungi although members of the same species appear to have lost, or gained the capability through evolution (see Varga *et al.*, 2003) or possibly preservation (Santos *et al.*, 2002). The effect of preservation regimes on the gene product could usefully be determined. It would be advantageous to test cultures which are assumed to be patulin producers immediately before preservation for the presence of the IDH gene. Traditional morphological identifications of fungi are not predictive of even the potential for patulin production capabilities in some cases. It is important to identify all sources of patulin contamination reliably from susceptible commodity systems. To determine if a commodity contains a particular mycotoxin biochemical analysis is required. An isolated fungus may not even have the capability of producing the toxin if identified by morphological (or other) characters, as demonstrated here.

Table 2. Possession of the isoepoxydon dehydrogenase (IDH) gene and patulin detection combining present data, Paterson *et al.* (2003) and Varga *et al.* (2003)

Species	Number of strains with traits		
	A	B	C
Aspergillus clavatus	4	3	3
A. giganteus	1	1	1
Penicillium brevicompactum	4 (1)[2] (7)[3]	16 (7)[3]	12 (18)[1]
P. expansum	15 (49)[2] (10)[3]	(10)[3]	1
P. paxilli	2 (1)[3]	(1)[3]	(1)[1]
P. roqueforti	15 (1)[2] (1)[3]	(1)[3]	
P. aurantiogriseum	1	–	2(1)[1]
P. griseofulvum	1	–	1
P. janczewskii	(1)[2]	–	6 (1)[1]
A. longivesica	1	–	–
A. pallidus	2	–	–
Byssochlamys nivea	1	–	–
P. griseofulvum var. *dipodomyicola*	1	–	–
A. clavatonanica	–	1	–
P. glandicola var. *glaucovenetum*	–	1	–
P. hirsutum	–	1	–
A. flavus	–	–	(1)[1]
A. rhizopodus	–	–	1
B. fulva	–	–	2
Ganoderma sp.	–	–	1
Neocarpenteles acanthosporus	–	–	1
Paecilomyces variotii	–	–	2
P. canesensens	–	–	(1)[1]
P. citrinum	–	–	1 (3)[1]
P. corylophilum	–	–	(1)[1]
P. chrysogenum	–	–	(1)[1]
P. glabrum	–	–	4
P. gladicola	–	–	1
P. janthenellum	–	–	2
P. melini	–	–	2
P. novae-zeelandiae	–	–	1
P. restrictum	–	–	1
P. simplicissimum	–	–	19 (5)[1]
P. spinulosum	–	–	(1)[1]
P. selandiae	–	–	1
P. waksmanii	–	–	2

A = IDH, Patulin positive; B = IDH positive, Patulin negative; C = IDH, patulin negative. ()[1] IDH negative, patulin not tested but assumed negative. ()[2] Patulin positive; IDH not tested but assumed positive. ()[3] IDH positive, patulin not tested but could have been be positive or negative, hence the same data appears in columns A and B.

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